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PHARMACOLOGICAL SPARING OF PROTEIN  
IN BURN INJURY  
ANNUAL REPORT

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<p>To test the responsiveness of protein kinetics to hyperinsulinemia and the maximum biological effectiveness of insulin to stimulate glucose uptake in severely burned and septic patients, isotopes of glucose, leucine and urea were infused to determine the kinetics of these substrates in 8 severely burned and 8 septic patients.</p> <p>Compared to the severely burned patients glucose uptake in the septic patients was depressed by 50% (<math>12.5 \pm 0.7</math> mg/kg·min in burns, <math>6.5 \pm 1.2</math> mg/kg·min in sepsis versus <math>14.0 \pm 1.3</math> mg/kg·min in bedrested controls).</p> <p>The basal rate of appearance (<math>R_a</math>) of leucine in the septic patients <math>4.08 \pm 0.22</math> umol/kg·min was similarly elevated as in the burn patients, <math>5.15 \pm 0.24</math> umol/kg·min when compared to controls <math>2.78 \pm 0.16</math> umol/kg·min indicating a marked stimulation in the absolute rate of protein breakdown in both groups of patients. In response to the hyperinsulinemic clamp there were significant reductions (<math>p &lt; 0.01</math>) in leucine <math>R_a</math> in both groups of patients to <math>3.39 \pm 0.14</math> umol/</p>					
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kg·min in burns,  $2.99 \pm 0.19$  umol/kg·min in sepsis and to  $1.60 (\pm 0.05)$  umol·kg<sup>-1</sup>·min<sup>-1</sup> in controls suggesting no impairment in the ability of insulin to restrain the absolute protein breakdown rate in both burned and septic patients. As a consequence of these reductions in the absolute rates of protein breakdown, there were marked reductions in the plasma concentrations of all amino acids. (500)

The basal rate of leucine oxidation was similarly elevated in burns  $1.6 \pm 0.14$  umol/kg·min and sepsis  $1.25 \pm 0.15$  umol/kg·min when compared to control values,  $0.61 \pm 0.07$  umol/kg·min, indicating a marked stimulation of net protein catabolism in both groups of patients. The insulin infusion elicited similar and significant ( $p < 0.01$ ) reductions in leucine oxidation in both groups of patients, which were not different from the response in the control subjects. Urea production rate (Ra) decreased to the same extent in both groups of patients in response to the insulin infusion, from  $8.19 \pm 0.92$  to  $7.01 \pm 0.66$  umol/kg·min in burns and from  $6.23 \pm 1.15$  to  $5.49 \pm 0.99$  umol/kg·min in sepsis (Table 4). In spite of the significant reductions in leucine Ra and oxidation, and urea Ra in the two groups of patients, when compared to the normal control values, the hyperinsulinemia failed to normalize any of these kinetic parameters.

We conclude that (1) there is a defect in the ability of insulin to stimulate glucose uptake in a normal way in septic but not in burn patients. (2) There is no impairment in the maximal effectiveness of insulin to suppress absolute and net protein breakdown rates and factors other than insulin resistance are responsible for stimulating the net protein catabolism of severe burn injury and sepsis.

FOREWORD

For the protection of human subjects the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

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In the past year we have used the hyperinsulinemic euglycemic clamp technique to investigate the role of insulin resistance in mediating the deranged protein kinetic response to severe burn injury in 8 burned patients. Because there is evidence in the literature to suggest that the "insulin resistance" of sepsis may be different from that of severe trauma uncomplicated by infection, we further tested the effect of hyperinsulinemia on glucose, leucine and urea kinetics in 8 septic patients.

Compared to the severely burned patients glucose uptake in the septic patients was depressed by 50% ( $12.5 \pm 0.7$  mg/kg $\cdot$ min in burns,  $6.5 \pm 1.2$  mg/kg $\cdot$ min in sepsis versus  $14.0 \pm 1.3$  mg/kg $\cdot$ min in bedrested controls). Because endogenous glucose production was totally suppressed in both groups of patients we concluded that there is a defect (peripheral) in the ability of insulin to stimulate glucose uptake in a normal way in septic but not in burn patients. The basal rate of appearance (Ra) of leucine in the septic patients  $4.08 \pm 0.22$   $\mu$ mol/kg $\cdot$ min was similarly elevated as in the burn patients,  $5.15 \pm 0.24$   $\mu$ mol/kg $\cdot$ min when compared to control values  $2.78 \pm 0.16$   $\mu$ mol/kg $\cdot$ min (Tables 1 and 2) indicating a marked stimulation in the absolute rate of protein breakdown in both groups of patients. The response to the hyperinsulinemic clamp was similar to the response observed in controls, as there were significant reductions ( $p < 0.01$ ) in leucine Ra in both groups of patients to  $3.39 \pm 0.14$   $\mu$ mol/kg $\cdot$ min in burns,  $2.99 \pm 0.19$   $\mu$ mol/kg min in sepsis and to  $1.60 \pm 0.05$   $\mu$ mol $\cdot$ kg $^{-1}\cdot$ min $^{-1}$  in controls, (Tables 1 and 2) suggesting no impairment in the ability of insulin to restrain the absolute protein breakdown rate in both burned and septic patients. As a consequence of these reductions in the absolute rates of protein breakdown, there were marked reductions in the plasma concentrations of all amino acids (Table 3).

The basal rate of leucine oxidation was similarly elevated in burns  $1.6 \pm 0.14$   $\mu\text{mol/kg}\cdot\text{min}$  and sepsis  $1.25 \pm 0.15$   $\mu\text{mol/kg}\cdot\text{min}$  when compared to control values,  $0.61 \pm 0.07$   $\mu\text{mol/kg}\cdot\text{min}$ , indicating a marked stimulation of net protein catabolism in both groups of patients. The insulin infusion elicited significant reductions ( $p < 0.05$ ) in leucine oxidation in both groups of patients (Tables 1 and 2) which were not different from the response in controls (from  $0.61 \pm 0.01$  to  $0.32 \pm 0.01$   $\mu\text{mol/kg}\cdot\text{min}$ ) suggesting that there was no impairment in the ability of insulin to inhibit net protein catabolism in both burn and septic patients. This finding was further supported by the fact that urea production rate ( $R_a$ ) decreased to the same extent in both groups of patients in response to the insulin infusion, from  $8.19 \pm 0.92$  to  $7.01 \pm 0.66$   $\mu\text{mol/kg}\cdot\text{min}$  in burns and from  $6.23 \pm 1.15$  to  $5.49 \pm 0.99$   $\mu\text{mol/kg}\cdot\text{min}$  in sepsis (Table 4). In spite of the significant reductions in leucine  $R_a$  and oxidation, and urea  $R_a$  in the two groups of patients, when compared to the normal control values, the hyperinsulinemia failed to normalize any of these kinetic parameters, which still remained markedly elevated; suggesting that factors other than insulin resistance were responsible for mediating the deranged protein metabolism of burns and sepsis.

From studies by Clowes group in which the rate of uptake of substrates across the leg of septic and several traumatized patients (and pigs) were measured, it was suggested that there is a deficit in the supply of substrates for energy production in peripheral tissues. As a consequence they postulated that protein is broken down at a faster rate to supply amino acids as fuel in order to compensate for the decreased rate of uptake of glucose and free fatty acids. Because we now know that we can stimulate (normalize) glucose uptake in burn patients and reduce but not normalize protein breakdown we propose to

test the hypothesis of Clowes group "that the increase in protein breakdown was due to a shortage in peripheral energy supply", by further stimulating glucose oxidation during the hyperinsulinemic clamp and measuring its effect on leucine and urea kinetics. Glucose oxidation rate will be stimulated by administering dichloroacetate (DCA) during the hyperinsulinemic clamp to stimulate pyruvate dehydrogenase activity (which should divert more pyruvate towards the oxidative pathway as opposed to non-oxidative) thereby increasing the rate of glucose oxidation. Both burned and septic patients will be studied over the next quarter. If their hypothesis is correct the provision of more energy by the further stimulation of glucose oxidation by DCA administration should elicit a further reduction (or normalization) of leucine and urea kinetics.

TABLE 1  
RESPONSE OF LEUCINE KINETICS TO HYPERINSULINEMIC EUGLYCEMIC  
CLAMP IN BURNED PATIENTS (PROTOCOL 1)

Patient	Leucine Ra		Oxidation	
	Basal	Clamp	Basal	Clamp
	(umol•kg <sup>-1</sup> •min <sup>-1</sup> )			
A	4.10	2.65	1.64	0.70
B	6.18	3.79	1.79	0.57
C	5.49	3.43	1.94	1.25
D	4.93	3.11	1.98	0.79
E	4.67	3.46	1.12	1.07
F	4.81	3.71	1.98	1.48
G	5.18	3.19	1.12	1.12
H	5.83	3.76	1.22	1.04
Mean ± SEM	5.15±0.24	3.39±0.14 <sup>+</sup>	1.60±0.14	1.00±0.11 <sup>+</sup>

<sup>+</sup> Significantly different from basal value (p < 0.01).

TABLE 2  
RESPONSE OF LEUCINE KINETICS TO HYPERINSULINEMIC EUGLYCEMIC  
CLAMP IN SEPTIC PATIENTS (PROTOCOL 1)

Patient	Leucine Ra		Oxidation	
	Basal	Clamp	Basal	Clamp
	(umol·kg <sup>-1</sup> ·min <sup>-1</sup> )			
I	4.04	2.90	1.29	1.28
J	2.95	2.11	1.15	1.06
K	4.68	3.55	1.62	1.37
L	4.94	3.72	1.32	1.03
M	4.30	2.94	0.90	0.70
N	3.91	3.29	0.85	0.90
O	3.69	2.50	0.76	0.63
P	4.13	2.89	2.08	1.46
Mean ± SEM	4.08±0.22	2.99±0.19 <sup>+</sup>	1.25±0.15	1.06±0.11 <sup>+</sup>

<sup>+</sup> Significantly different from basal value, (p < 0.05).

TABLE 3

RESPONSE OF PLASMA AMINO ACID CONCENTRATIONS TO A HYPERINSULINEMIC  
EUGLYCEMIC CLAMP IN BURN AND SEPTIC PATIENTS (Values are Mean  $\pm$  SEM)

Amino Acid	Burn			Sepsis		
	Basal	Clamp	p*	Basal	Clamp	p
	$\mu\text{M}$	$\mu\text{M}$		$\mu\text{M}$	$\mu\text{M}$	
Aspartate	24 $\pm$ 2	16 $\pm$ 1.3	< 0.01	26 $\pm$ 3	21 $\pm$ 3	< 0.01
Glutamate plus Glutamine	296 $\pm$ 32	202 $\pm$ 20	< 0.01	410 $\pm$ 41	262 $\pm$ 33	< 0.01
Alanine	193 $\pm$ 21	130 $\pm$ 15	< 0.01	280 $\pm$ 42	205 $\pm$ 29	< 0.01
Glycine	141 $\pm$ 10	110 $\pm$ 4	< 0.01	177 $\pm$ 19	144 $\pm$ 16	< 0.01
Serine	79 $\pm$ 7	43 $\pm$ 4	< 0.01	76 $\pm$ 4	54 $\pm$ 5	< 0.01
Proline	144 $\pm$ 15	85 $\pm$ 8	< 0.01	227 $\pm$ 49	149 $\pm$ 29	< 0.01
Threonine	79 $\pm$ 7	38 $\pm$ 4	< 0.01	89 $\pm$ 8	56 $\pm$ 6	< 0.01
Methionine	25 $\pm$ 2	10 $\pm$ 1.3	< 0.01	35 $\pm$ 6	17 $\pm$ 3	< 0.01
Lysine	148 $\pm$ 6	96 $\pm$ 4	< 0.01	172 $\pm$ 19	124 $\pm$ 13	< 0.01
Histidine	56 $\pm$ 4	45 $\pm$ 3	< 0.05	96 $\pm$ 17	78 $\pm$ 14	< 0.01
Valine	191 $\pm$ 21	75 $\pm$ 9	< 0.01	179 $\pm$ 23	103 $\pm$ 22	< 0.01
Isoleucine	60 $\pm$ 9	21 $\pm$ 3	< 0.01	58 $\pm$ 4	21 $\pm$ 4	< 0.01
Leucine	127 $\pm$ 13	60 $\pm$ 6	< 0.01	144 $\pm$ 19	78 $\pm$ 14	< 0.01
Tyrosine	61 $\pm$ 5	29 $\pm$ 2	< 0.01	70 $\pm$ 5	44 $\pm$ 4	< 0.01
Phenylalanine	78 $\pm$ 4	54 $\pm$ 3	< 0.01	115 $\pm$ 5	86 $\pm$ 6	< 0.01
Arginine	69 $\pm$ 6	45 $\pm$ 6	< 0.01	79 $\pm$ 7	54 $\pm$ 6	< 0.01

\*Significance of difference from basal.

TABLE 4  
RESPONSE OF UREA KINETICS TO HYPERINSULINEMIC EUGLYCEMIC  
CLAMP IN BURNED AND SEPTIC PATIENTS (PROTOCOL 1)

Burn Patients	Urea Ra		Septic Patients	Urea Ra	
	Basal	Clamp		Basal	Clamp
	(umol•kg <sup>-1</sup> •min <sup>-1</sup> )				
A	10.15	8.51	I	2.87	2.97
B	8.47	7.46	J	2.79	2.42
C	7.15	6.69	K	10.78	8.93
D	13.35	10.02	L	10.16	9.50
E	6.37	5.26	M	8.18	6.91
F	8.24	8.04	N	3.01	2.50
G	6.82	5.76	O	5.68	4.64
H	4.97	4.34	P	6.39	6.06
Mean <u> </u> SEM	8.19 <u> </u> 0.92	7.01 <u> </u> 0.66 <sup>+</sup>		6.23 <u> </u> 1.15	5.49 <u> </u> 0.99 <sup>+</sup>

<sup>+</sup> Significantly different from basal value, ( $p < 0.05$ ).